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Indicators for the total duration of premenopausal endogenous estrogen exposure in relation to BMD

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BACKGROUND: Previous studies have shown that age at menopause is an important indicator of duration of endogenous estrogen exposure. The present study investigates whether combining more information on reproductive factors is useful in estimating individual total duration of exposure to endogenous estrogens. **METHODS:** Bone mineral density (BMD) was used as operational outcome. The study population consisted of 3476 white women living in Eindhoven, The Netherlands, aged 46–57 years, either pre- ($n = 2420$) or postmenopausal ($n = 1056$). BMD of the lumbar spine was measured by dual X-ray absorptiometry. Information on reproductive factors was obtained with questionnaires. **RESULTS:** The number of reproductive years explained 4.8% of the variance in BMD, while age at menopause alone accounted for 3.6%. Duration of lactation or oral contraceptive use did not add to the proportion of variance explained. The effect of reproductive years on BMD was stronger in older women. No significant associations with BMD were found for other reproductive variables. The number of miscarriages in premenopausal women ($\beta = 0.00760$, $SE = 0.00357$, $P = 0.03$) explained only 0.16% of the variance in BMD. **CONCLUSIONS:** We conclude that it is not necessary to use more reproductive factors besides age at menopause and menarche in determining total duration of endogenous estrogen exposure.

Key words: bone mineral density/estrogen/premenopausal/postmenopausal/reproductive factors

Introduction

Most of the epidemiologic research on the association between endogenous estrogens and cardiovascular disease (van der Schouw *et al.*, 1996; Jacobsen *et al.*, 1997), breast cancer (Verheul *et al.*, 2000; Zheng *et al.*, 2001), osteoporosis (Cumming and Klineberg 1993, 1998; Eisman, 1998; Jacobsen *et al.*, 1998) and colorectal cancer (Kampman *et al.*, 1997; Slattery *et al.*, 2001) has focused on individual reproductive factors. Age at menopause and age at menarche seem to be of overriding importance in determining total endogenous estrogen exposure, but factors like pregnancy, regularity and duration of the menstrual cycle, lactation and use of oral contraceptives also play a role.

As a measure of endogenous estrogen exposure, sex hormone levels in blood are not easily interpretable, because there is huge variation in hormone levels through the menstrual cycle and it is not clear which menstrual phase most adequately reflects premenopausal hormone exposure (Sowers *et al.*, 2003).

Bone mineral density (BMD) is considered a good outcome parameter, since it is generally accepted that

endogenous estrogens and BMD are strongly associated (Zhang *et al.*, 2001).

Consistent associations have been reported between BMD and either age at menarche (Kritz Silverstein and Barrett Connor 1993; Ito *et al.*, 1995), or age at menopause (Kritz Silverstein and Barrett Connor 1993; Ito *et al.*, 1995; Parazzini *et al.*, 1996), or the number of reproductive years (Vico *et al.*, 1992; Kritz Silverstein and Barrett Connor 1993; Ito *et al.*, 1995; Nguyen *et al.*, 1995; Grainge *et al.*, 2001). The combination of age at menopause and age at menarche in one measure appears to explain more of the variance in bone mineral density than either variable individually (Kritz Silverstein and Barrett Connor 1993). However, other reproductive factors are also associated with BMD and may therefore be useful in refining the assessment of endogenous estrogen exposure.

During pregnancy maternal estrogen levels markedly increase (Speroff *et al.*, 1989), which may have a beneficial effect on BMD. However, during pregnancy and lactation the demand of calcium is increased and some loss of bone

calcium is to be expected (Khastgir *et al.*, 1994). Several epidemiologic studies show a significantly increased bone density with increasing parity (Nguyen *et al.*, 1995; Tuppurainen *et al.*, 1995) while others do not (Hamed *et al.*, 1992b; Johansson *et al.*, 1993; Parazzini *et al.*, 1996). During lactation, estrogen production is suppressed (Rolland, 1995). This relative shortage of estrogen during one or more breast-feeding periods could theoretically lead to a lower bone density after the breastfeeding period. During breastfeeding loss of bone mineral has been noted in several sites in the peripheral skeleton. Some studies have shown that the loss in bone mineral is almost completely restored 6 to 12 months after weaning and with the return of normal menses (Eisman, 1998). In other studies a negative effect (Lissner *et al.*, 1991) or no effect (Tuppurainen *et al.*, 1995; Parazzini *et al.*, 1996; Grainge *et al.*, 2001) of lactation on BMD has been found. It is, however, not possible to distinguish between the potential effect of a low estrogen exposure and the effect of an increased demand for calcium.

Studies on the relationship between oral contraceptives and bone density have shown conflicting results (Lindsay *et al.*, 1986; Recker *et al.*, 1992; Murphy *et al.*, 1993; DeCherney, 1996; Vessey *et al.*, 1998; Grainge *et al.*, 2001). As oral contraceptives, like hormone replacement therapy, are a source of exogenous estrogens, one would expect them to have a positive effect on bone density, although the dose and type of estrogens are different from those used in hormone replacement therapy.

The objective of this study is to investigate whether combining information on these reproductive factors is useful in estimating individual total duration of exposure to endogenous estrogen. For this purpose, we investigate to what extent the variation in BMD pre- and post-menopause can be explained by differences in total endogenous estrogen exposure and whether a more refined measure of endogenous estrogen exposure adds to the predictive value for BMD of the single factor 'age at menopause'.

Subjects and methods

Study population

The screening of women for the Eindhoven Perimenopausal Osteoporosis Study (EPOS) was conducted between September 1994 and September 1995. All responders gave their written informed consent and the study was approved by two medical ethics committees. The study population has been described in detail before (Smeets-Goevaers *et al.*, 1998). For the present study, information was available on 6700 Dutch women living in the city of Eindhoven and born between 1938 and 1948. Two hundred and fifty one women were non-Caucasian and were excluded from the current analysis. Women with uncertain menopausal status or age at menopause were excluded ($n = 2942$) e.g. women who used hormone replacement therapy ($n = 1608$) and those who reported hysterectomy, oophorectomy or both ($n = 775$). Thirty women were excluded because they did not provide accurate information on reproductive factors and one woman was excluded because BMD could not be measured.

Women who had not had natural menses for 12 months or more at the time of measurement were defined postmenopausal. Age at menopause was defined as age at the last menstrual period. The population for analysis comprised 2420 pre- and 1056 post-menopausal women.

Data collection

BMD of the lumbar spine was measured by dual X-ray absorptiometry (DXA; SA Hologic Europe, Brussels). Height and weight were measured at the screening of women before enrolment. Body mass index was calculated as weight in kilograms divided by the square of height in meters. Information about age at menopause, age at menarche, number of live born children, number of miscarriages and stillborn children, total duration of breastfeeding, length of the menstrual cycle, total duration of oral contraceptive use and smoking habits was assessed by a questionnaire. Information about the duration and regularity of the menstrual cycle was available in only a subset of our data. In the postmenopausal group, the subset was too small ($n = 40$) to draw any conclusions, but in the premenopausal group the number of subjects was reasonably large ($n = 1372$). In this subset of the premenopausal group, we examined the length of the menstrual cycle in relation to BMD. Smoking habits were recorded in five categories: never, current ≥ 10 cigarettes/day, current < 10 cigarettes/day, past ≥ 10 cigarettes/day and past < 10 cigarettes/day.

Measures of estrogen exposure

An estimation of the total duration of exposure to endogenous estrogens in the postmenopausal group was obtained as follows: for each woman age at menarche, which indicates the start of endogenous estrogen production, was subtracted from age at menopause, which indicates the end of estrogen production by the ovaries; the total number of reproductive years. As there is negligible exposure to estrogen in a lactating woman, the total duration of breastfeeding was subtracted. Similarly, the total duration of oral contraceptive use was subtracted from the duration of exposure to endogenous estrogens. The oral contraceptives that were used before 1971–1975 contained a very high dose of synthetic estrogen and suppressed endogenous estrogen production.

To investigate the added value of each component of the total duration of endogenous estrogen exposure, we calculated different measures of exposure varying from the most elaborate calculation, containing all components, to a 'calculation' consisting only of age at menopause.

In the premenopausal group, it was not possible to design a cumulative measure for estrogen exposure in the same way as we did in the postmenopausal group. The use of age at measurement of BMD instead of menopausal age for the calculation of the number of reproductive years is conceptually not right, as BMD decreases with chronological age and increases with age at last menses. Therefore, age at menarche, adjusted for age, was used as a measure of cumulative exposure to endogenous estrogen in the premenopausal group. The adjustment for age has to be made because older people have been exposed to endogenous estrogen for

Table I. Characteristics of the 3476 Dutch women included in the study^a

Characteristics	Total group <i>n</i> = 3476 ^b	Premenopausal women <i>n</i> = 2420	Postmenopausal women <i>n</i> = 1056
Spine BMD, g/cm ²	1.013 (0.002)	1.045 (0.003)	0.938 (0.004)
Age, years	50.7 (0.04)	49.9 (0.04)	52.5 (0.07)
Age at menarche, years	13.3 (0.03)	13.3 (0.03)	13.4 (0.05)
Number of live-born children	1.9 (0.02)	1.9 (0.02)	1.9 (0.04)
Number of miscarriages/stillborn children	0.3 (0.01)	0.3 (0.01)	0.4 (0.02)
Total duration of lactation, months	1.7 (0.04)	1.7 (0.05)	1.8 (0.08)
Use of oral contraceptives ever, %	74.6	77.1	68.8
Total duration, for users, of oral contraceptive use, months	77.3 (1.11)	75.6 (1.33)	81.8 (2.05)
Length of the menstrual cycle, days ^c	–	27.2 (0.00)	–
Body mass index, kg/m ²	25.3 (0.08)	25.5 (0.09)	25.1 (0.13)
Ever smoked, %	66.6	66.1	67.8
Age at menopause, years	–	–	48.3 (0.13)
Number of reproductive years	–	–	34.9 (0.14)
Time since menopause, years	–	–	4.2 (0.11)

^aMean (SE) or %.^bThe number of women given for each group is the maximum number of observations used in the calculations.^cThis variable was studied in 1372 women.

a longer period of time, while at the same time age itself has an independent, negative effect on BMD.

Data analysis

BMD measurements were not sufficiently normally distributed to be modeled in a linear regression model and were included after a natural logarithm transformation. Since the observed changes in BMD with the total duration of exposure to endogenous estrogens were not large, the resulting coefficients for the transformed BMD may be interpreted as the relative change in the untransformed BMD.

The associations between the various estimates of premenopausal endogenous estrogen exposure and BMD in the postmenopausal group were studied by means of linear regression analysis in uni- and multivariate models. To determine whether 'total duration of exposure to endogenous estrogens' is a better predictor for BMD than 'age at menopause' we compared the proportion of variance in BMD explained by the linear regression models. BMD and duration of estrogen exposure were analyzed as continuous variables. In the multivariate analyses, adjustments were made for age, body mass index and smoking. Furthermore, the crude and adjusted relationships between the separate reproductive factors and BMD were determined in both the pre- and the postmenopausal group.

Presence of effect modification of the association between endogenous estrogen exposure and BMD by other variables was assessed by adding interaction terms to the linear regression model. If effect modification was present, the variables were divided in two categories with the median as cut-off point. The adjusted estimates are presented for each category of these variables.

All analyses were performed using the Statistical Analysis System, version 6.11 (SAS Institute, Cary, NC).

Results

General characteristics of the study population are shown in Table I. The average BMD in the premenopausal women was

1.045 g/cm² (SE 0.003) and in the postmenopausal women 0.938 g/cm² (SE 0.004). The mean age at menopause in the postmenopausal women was 48.3 (SE 0.13) years and mean number of reproductive years was 34.9 (SE 0.14). There were no differences between age at menarche in the premenopausal group and age at menarche in the postmenopausal group.

Tables II and III show the adjusted estimates of the relationship between the individual reproductive factors, body mass index, age and the time since the menopause and BMD. The adjusted relationship between BMD and age at menarche, which we used as a cumulative measure for endogenous estrogen exposure in the premenopausal group, was not significant. No significant relationships were found between BMD and the number of live born children, the total duration of breastfeeding and the total duration of use of oral contraceptives. The variance in BMD explained by these reproductive factors was extremely small compared to that of age at menopause. In the premenopausal group, but not in

Table II. Adjusted estimates^a for the relationship between bone mineral density and several reproductive variables in 2420^b Dutch premenopausal women

Variable	β (SE)	<i>P</i>	R ² adj
Body mass index, kg/m ²	0.00623 (0.00058)	0.0001*	0.0536
Age, years	–0.00570 (0.00124)	0.0001*	0.0536
Age at menarche, years	–0.00237 (0.00158)	0.1349	0.0548
Number of live-born children	–0.00006 (0.00254)	0.9823	0.0532
Number of miscarriages	0.00760 (0.00357)	0.0334*	0.0550
Total duration of use of oral contraceptives, years	–0.00012 (0.00048)	0.8097	0.0539
Total duration of lactation, months	0.00107 (0.00104)	0.3044	0.0536
Length of the menstrual cycle ^c	–0.00132 (0.00098)	0.1779	0.0635

^aBody mass index adjusted for age and smoking, age adjusted for body mass index and smoking. All other variables adjusted for age, body mass index and smoking.^bThe number of women is the maximum number of observations used in the calculations.^cThis variable was studied in 1372 women.**P* < 0.05.

Table III. Adjusted estimates^a for the relationship between bone mineral density (g/cm²) and several reproductive variables in 1056^b Dutch postmenopausal women

Variable	β (SE)	<i>P</i>	R ² adj
Body mass index, kg/m ²	0.00882 (0.00101)	0.0001*	0.0775
Age, years	-0.00057 (0.00190)	0.7669	0.0775
Time since the menopause	-0.00893 (0.00123)	0.0001*	0.1213
Age at menarche, years	-0.00509 (0.00264)	0.0542	0.0799
Number of live-born children	-0.00274 (0.00388)	0.4809	0.0771
Number of miscarriages	0.00230 (0.00555)	0.6793	0.0768
Total duration of use of oral contraceptives, years	0.00037 (0.00079)	0.6422	0.0768
Total duration of lactation, months	-0.00315 (0.00168)	0.0608	0.0797

^aBody mass index adjusted for age and smoking, age adjusted for body mass index and smoking. All other variables adjusted for age, body mass index and smoking.

^bThe number of women given for each group is the maximum number of observations used in the calculations.

**P* < 0.05.

the postmenopausal group, a significant relationship with BMD was found for the number of miscarriages ($\beta = 0.00760$, SE = 0.00357, *P* = 0.03), although the proportion of variance in BMD explained by this variable was only 0.16% (not shown in Table).

Body mass index was significantly associated with BMD in both the pre- and the post-menopausal group, although its effect was more pronounced after menopause. In the present study, body mass index appeared to be the strongest confounder of the relationship between reproductive factors and BMD.

Chronological age, crude as well as corrected for BMI and smoking, was not significantly associated with BMD in the postmenopausal women. In the premenopausal women, an effect of chronological age was clear but did not explain a large proportion of variance in BMD ($R^2 = 0.0047$; not shown in Table). In the postmenopausal group a relatively strong inverse association with BMD was found for the time since the menopause. The time since the menopause explained 4.6% of the variance in BMD (not shown in Table).

We repeated our analyses in the subset of the premenopausal group for which the length of the menstrual cycle was known. Length of the menstrual cycle was not significantly associated with BMD and adding this variable to the multivariate model did not materially alter the results.

The crude associations between various measures for total duration of endogenous estrogen exposure and BMD in the postmenopausal women are shown in Table IV. The models comprising duration of breastfeeding and/or duration of use of oral contraceptives did not add to the predictive value of the number of reproductive years ($\beta = 0.00708$, *P* = 0.0001, $R^2 = 0.048$). The number of reproductive years was a better predictor for BMD after the menopause than age at menopause ($\beta = 0.00645$, *P* = 0.0001, $R^2 = 0.036$) or age at menarche ($\beta = -0.00937$, *P* = 0.0001, $R^2 = 0.011$; unadjusted, not shown in Table) separately.

In both the pre- and the post-menopausal group, none of the reproductive factors duration of breastfeeding, number of children, number of miscarriages and total duration of oral contraceptive use confounded the relationship between age at menopause, age at menarche or the measures for cumulative endogenous estrogen exposure and BMD.

The effect of the number of reproductive years appeared to increase with chronological age (*P* for interaction 0.02) and to decrease with time since the menopause (*P* for interaction 0.02). In Table V, the coefficients for the number of reproductive years in the postmenopausal group, adjusted for age, body mass index and smoking, are presented in strata of age and time since the menopause. The relationship between the number of reproductive years and BMD was stronger in the women over 52.6 years of age compared to the women younger than 52.6 years of age. However, this relationship was weaker in the women whose last menses was more than 2.9 years ago compared to the women whose last menses was less than 2.9 years ago.

Discussion

In this population of 3476 Dutch women, total endogenous estrogen exposure predicted BMD levels in pre- and post-menopausal women. However, the inclusion of more reproductive variables in the calculation of duration of exposure to endogenous estrogens did not add to the predictive value for BMD of the number of reproductive years, calculated as age at menopause minus age at menarche. The relationship between the number of reproductive years and BMD in post-menopausal women differed with age and the number of years since the menopause. No significant associations with BMD were found for age at menarche, parity, duration of

Table IV. Associations between different measures of total duration of endogenous estrogen exposure and bone mineral density (g/cm²) in 1056^a Dutch postmenopausal women

Measure of total duration of endogenous estrogen exposure	Coefficient of linear regression (SE) ^b	R ² adj
Age at menopause – age at menarche – total duration of lactation – duration of oc use	0.00276 (0.00060)	0.019
Age at menopause – age at menarche – duration of oc use	0.00275 (0.00060)	0.019
Age at menopause – age at menarche – total duration of lactation	0.00715 (0.00096)	0.049
Age at menopause – age at menarche	0.00708 (0.00096)	0.048
Age at menopause	0.00645 (0.00101)	0.036

^a1056 is the maximum number of observations used in the calculations. Occasionally there are missing data, but this never exceeds 2%.

^b*P* < 0.001 for all estimates.

oc = oral contraceptive.

Table V. Adjusted estimates for the relationship between bone mineral density and number of reproductive years in 1048^a Dutch postmenopausal women, presented by age and time since the menopause

Subgroup	<i>n</i>	β (SE) ^b for number of reproductive years	<i>P</i>
Age			
< 52.6	523	0.00736 (0.00149)	0.0001
≥ 52.6	525	0.00934 (0.00171)	0.0001
Time since the menopause			
< 2.9	523	0.00682 (0.00351)	0.0524
≥ 2.9	525	0.00610 (0.00152)	0.0001

^aOf the 1056 women in the postmenopausal group, data on number of reproductive years were missing for two observations and data on covariates for four observations.

^bAll estimates are adjusted for age, body mass index and smoking.

breastfeeding, duration of oral contraceptive use and length of the menstrual cycle.

To appreciate these results, some methodological issues need to be addressed. The data on reproductive factors were based on self-report and thus depend on the correctness of the women's recollection. Although it has been reported that women's recall of their reproductive history is sufficiently accurate to detect most associations between risk factors and osteoporosis (Cummings, 1991), the possibility of recall errors cannot be excluded. However, such recall errors are likely to be random with respect to BMD and, if present, will only lead to an underestimation of the effect by biasing estimates towards the null.

As only women who did not use hormone replacement therapy were included in this study, selection may be present. The women who did use hormone replacement therapy may have been the women with a lower than average BMD, which may have led to a reduced variability in BMD in the remainder of the population.

The number of reproductive years explained only 4.8% of the variance in BMD. However, as it has been postulated that genetic factors may account for up to 80% of the variance in BMD (Health Council of The Netherlands: Committee on Osteoporosis, 1998; Rubin *et al.*, 1999), duration of exposure to endogenous estrogen explains a material proportion of the remaining 20%. Factors such as physical activity, body mass index, smoking, and calcium intake may also play a role in determining BMD (Guthrie *et al.*, 1996; Snelling *et al.*, 2001).

The effect of body mass index on BMD and duration of exposure to endogenous estrogens in our population is difficult to interpret. A high body mass index is associated with more adipose tissue. Adipose tissue is capable of converting androstendione and testosterone into estrogens and continues doing so after menopause. At the same time, a high body mass index means a higher physical load on the bones, which may increase bone mass (Centraal begeleidingsorgaan voor de intercollegiale toetsing, 1992). The effect of both processes cannot be investigated separately. However, it is likely that obese people are subject to a lower level of physical activity (Martinez-Gonzalez *et al.*, 1999), which may compensate for the increased weight the bones have to carry. The net effect of these mechanisms is difficult to determine.

To our knowledge, we are the first to examine more refined models of duration of endogenous estrogen exposure in relation to BMD. However, a relationship between BMD and the number of reproductive years has been reported previously in several studies (Kritz Silverstein and Barrett Connor 1993; Melton III *et al.*, 1993; Ito *et al.*, 1995; Nguyen *et al.*, 1995; Grainge *et al.*, 2001). To date, only one study examined the association between number of reproductive years and hip fracture mortality. In a prospective study in Norway with over 60000 women followed up for 29 years, a mortality rate ratio of 0.5 in women with more than 38 reproductive years compared to women with fewer than 30 reproductive years was found (Jacobsen *et al.*, 1998). This finding suggests that the positive effect of the duration of the reproductive period on BMD is apparent in the risk of fractures as well.

We found an increase in BMD of 7.4% per 10 years of endogenous estrogen exposure in women aged <52.6 years ($P = 0.0001$) and 9.3% in women aged ≥52.6 years ($P = 0.0001$). Although other studies reported effects of similar magnitude, the difference in effect with increasing age has not been mentioned previously. In our population, higher age meant a more marked effect of the number of reproductive years on BMD. However, the strength of the association decreased with increasing time since the menopause. This is surprising, for attenuation of the effect both with age and with the number of years since the menopause, which reflects the longer time since exposure to high levels of endogenous estrogens, would be more conceivable. In order to elucidate these results, we calculated Pearson correlation coefficients for the two variables. This coefficient was -0.092 ($P = 0.0028$), indicating that age and the time since menopause were inversely correlated. In this post-menopausal population, it appeared, the older women were also the ones whose menopause came at an older age. We have to keep in mind, however, that although the correlation is significant, the coefficient is also very small. Adjustment for both age and the time since the menopause in one linear regression model is not possible, as there is a perfect co-linearity between age, age at menopause, and time since the menopause.

A lack of a clear effect on BMD for age at menarche is supported by some authors (Johansson *et al.*, 1993; Parazzini *et al.*, 1996) but not all (Ito *et al.*, 1995; Tuppurainen *et al.*, 1995). However, in our population the relationship is significant when the groups are combined and the number of women is larger. It seems to be a small effect that can only be demonstrated in a large group of people.

In the premenopausal group, but not in the postmenopausal group, a significant relationship with BMD was found for the number of miscarriages. A possible explanation for the positive effect of the number of miscarriages is, that a miscarriage exposes a woman to the same high levels of endogenous estrogens as a successful pregnancy but without an effect of a negative calcium balance. In two previous studies that investigated the number of abortions, no effect was found on BMD (Hamed *et al.*, 1992a) or hip fractures (Parazzini *et al.*, 1996).

In both the pre- and the post-menopausal group, no significant relationships with BMD were found in

uni- and multivariate analyses for the total duration of lactation, the number of live born children and the total duration of use of oral contraceptives. Although the number of children varied between 0 and 9, half of the women in both groups had just one or two children. The absence of effect found in our population may therefore be due to lack of variation in this variable. This might also apply for the duration of lactation. Furthermore, no data on whether breastfeeding was complete or incomplete were available.

The absence of effect for total duration of use of oral contraceptives cannot be attributed to small variation in this variable, as the range was quite wide. However, the effect of oral contraceptives on bone mass may be related to the specific formulation and dose (DeCherney, 1996). The current oral contraceptives have a much lower dose of estrogen and do not fully suppress the menstrual cycle. Considering their age, the women in this study may have been users of first, second or both generations of oral contraceptives. Thus, it is most likely that the suppression of endogenous cycles during the use of oral contraceptives was not complete. Furthermore, the use of oral contraceptives means the (partial) substitution of endogenous by exogenous estrogen exposure. Both are expected to have a positive effect on BMD and therefore it is possible that although oral contraceptives might suppress the endogenous cycle, the net effect on BMD is zero.

In this study we investigated only the effect of the duration of exposure to endogenous estrogens and did not take the intensity of exposure into account, such as during pregnancy, when maternal estrogen levels are much higher than during the normal menstrual cycle. However, the lack of effect found for pregnancy does not support the existence of an intensity effect of endogenous estrogens on BMD. During pregnancy the increased levels of serum estradiol are accompanied by an increased demand for calcium. It is conceivable that this results in a net effect of pregnancy that approaches zero.

Studies on the use of exogenous estrogens do indicate an effect of intensity, as dose-related effects of estrogens on bone density have been reported (DeCherney, 1996; Speroff *et al.*, 1996). In general it appears that the effect of hormone replacement therapy on bone is much larger than that of endogenous estrogen (Speroff *et al.*, 1996; The writing group for the PEPI trial, 1996).

In conclusion, this study showed that it is not necessary to include other reproductive factors than age at menopause and age at menarche in determining the total duration of endogenous estrogen exposure in both pre- and post-menopausal women.

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